

Please delete the paragraph that appears at page 3, lines 27-31, and substitute the following paragraph in place thereof.

C2
-- In particular embodiments, the isolated protein has an amino acid sequence as set forth in any one of FIGS. 5 to 9 (SEQ ID NOS: 23 to 27) which are examples of "*modified NhhA polypeptides of the invention*". In FIGS. 14A-14G (SEQ ID NOS: 33 to 39) further examples are provided of "mature" polypeptides predicted to result of removal of N-terminal signal sequences.

Please delete the paragraph that appears at page 6, line 25, through page 7, line 7, and substitute the following paragraph in place thereof.

C3
-- Table 1: Identification of amino acids of the conserved regions (C1, C2, C3, C4 and C5) and variable regions (V1, V2, V3 and V4) of an NhhA polypeptide from each of ten (10) indicated strains of *N. meningitidis*. Relevant SEQ ID NOS are also indicated. Column 1 = strain designation. SEQ ID NOS: 1-9 were previously described in copending application WO99/31132; the sequences of NhhA and *nhhA* of strain Z2491 were obtained from the database of the Wellcome Trust/Sanger Institute genomic sequencing project for *N. meningitidis*; column 2 = amino acid numbering of C1 region; column 3 = amino acid numbering of V1 region; column 4 = amino acid numbering of C2 region; column 5 = amino acid numbering of V2 region; column 6 = amino acid numbering of C3 region, column 7 = amino acid numbering of V2 region; column 8 = amino acid numbering of C4 region; column 9 = amino acid numbering of V4 region; column 10 = amino acid numbering of C5 region. Note that the amino acid numbering of the consensus sequence (SEQ ID NO: 11) is also indicated.

Please delete the paragraph that appears at page 7, lines 9-17, and substitute the following paragraph in place thereof.

C4
-- FIG. 1 (comprising FIGS. 1A-1E): Amino acid sequence alignments of NhhA polypeptide amino acid sequences from ten (10) *N. meningitidis* strains (SEQ ID NOS: 1-10) together with consensus sequence (SEQ ID NO: 11). Strain names and polypeptide sequences used in this alignment correspond to the strain names and SEQ ID NOS in column 1 of Table 1.

Amino acids are indicated by standard single letter abbreviations. Consensus amino acids are shown only where residues are completely conserved. Conserved regions (double underlined, labeled C1, C2, C3, C4, C5) and variable regions (single underlined, labeled V1, V2, V3, V4) are indicated under the consensus sequence. --

Please delete the paragraph that appears at page 7, lines 18-21, and substitute the following paragraph in place thereof.

C 5
--FIG. 2 (comprising FIGS. 2A-2H): Nucleotide sequence alignment of *nhhA* nucleic acids from ten (10) *N. meningitidis* strains, which sequences encode the amino acid sequences of FIG. 1. Regions C1, C2, C3, C4, C5 and V1, V2, V3, V4 are as described in FIG. 1 and Table 1. --

Please delete the paragraph that appears at page 8, line 28, through page 9, line 4, and substitute the following paragraph in place thereof.

C 6
--FIG. 10 (comprising Figures 10A and 10B): Amino acid sequence alignments of wild type and NhhA deletion mutant polypeptide sequences. These polypeptides were produced as described in Example 2, Example 3, Example 4 and Example 5. Amino acids are indicated by the one letter abbreviation. Conserved regions labeled C1, C2, C3, C4 and C5 corresponding to those defined in Table 1 and FIG. 1 are indicated by double underlining of full length sequences from H41 and PMC21, and variable regions labeled V1, V2, V3, V4 corresponding to those defined in Table 1 and FIG. 1 are indicated by single underlining of full length sequences from H41 and PMC21. --

Please delete the paragraph that appears at page 42, line 19, through page 43, line 4, and substitute the following paragraph in place thereof.

C 7
--The resulting plasmid, pIP52(PMC21), was linearized by restriction digestion and used to transform *N. meningitidis* strain 7G2 using the method described by Janik *et al*, 1976, Journal of Clinical Microbiology 4:71. Transformants were selected by overnight incubation at 37 °C in 5% CO₂ on solid media containing 100 µg/ml kanamycin. Kanamycin resistant colonies were selected, subcultured overnight and screened for over-expression of

NhhA polypeptide by separating total cell proteins electrophoretically on 10% SDS-PAGE followed by transfer to nitrocellulose membrane using a Semi-Dry Blotter (BioRad). The membrane was then incubated sequentially with rabbit anti-NhhA sera (as described in International Publication WO99/31132) and alkaline-phosphatase conjugated anti-Rabbit IgG (Sigma) before colorimetric detection with NBT/BCIP (Sigma). One clone was isolated which expressed NhhA polypeptide at a higher level compared with the parental strain (FIG. 11). Analysis of the predicted amino acid sequence using the computer program SIGCLEASE (part of the eGCG suite of programs hosted at the World Wide Web site of the Australian National Genomic Information Service {ANGIS}) indicates that the first 51 amino acids will be cleaved to produce the mature polypeptide (FIG. 14; SEQ ID NO: 33). --

Please delete the paragraph that appears at page 43, lines 9-19, and substitute the following paragraph in place thereof.

C8 -- The NhhA protein encoded by the *nhhA* gene of *N. meningitidis* strain H41 was over expressed using the same methods as described in Example 2. This created a recombinant nucleic acid expression construct (open reading frame shown in SEQ ID NO: 13) which encodes a polypeptide of 591 amino acids as shown in SEQ ID NO: 2. In this example the resulting plasmid pIP52(H41) was linearized, and transformed into *N. meningitidis* strain 7G2. Kanamycin resistant colonies were analysed and one was chosen which when examined by Western immunoblot, demonstrated overexpression of NhhA. (FIG. 11). Analysis of the predicted amino acid sequence using the computer program SIGCLEASE (part of the eGCG suite of programs hosted at the World Wide Web site of the Australian National Genomic Information Service {ANGIS}) indicates that the first 51 amino acids will be cleaved to produce the mature polypeptide (FIG. 14; SEQ ID NO: 34). --

Please delete the paragraph that appears at page 44, line 29, through page 45, line 4, and substitute the following paragraph in place thereof.

C9 -- Analysis of the predicted amino acid sequence using the computer program SIGCLEASE (part of the eGCG suite of programs hosted at the World Wide Web site of the Australian National Genomic Information Service {ANGIS}) indicates that the first 51 amino

acids will be cleaved to produce the mature polypeptide (FIG. 14; SEQ ID NO: 35). To confirm the presence of a cleavable signal sequence and to confirm the identity of the over expressed protein, outer membrane proteins were semi-purified by isolating the fraction that is insoluble in the detergent sarkosyl. /--

Please delete the paragraph that appears at page 46, lines 17-20, and substitute the following paragraph in place thereof.

C¹⁰ --] Analysis of the predicted amino acid sequence using the computer program SIGCLEASE (part of the eGCG suite of programs hosted at the World Wide Web site of the Australian National Genomic Information Service {ANGIS}) indicates that the first 51 amino acids will be cleaved to produce the mature polypeptide (FIG. 14; SEQ ID NO: 36). /--

Please delete the paragraph that appears at page 48, lines 13-16, and substitute the following paragraph in place thereof.

C¹¹ --] Analysis of the predicted amino acid sequence using the computer program SIGCLEASE (part of the eGCG suite of programs hosted at the World Wide Web site of the Australian National Genomic Information Service {ANGIS}) indicates that the first 51 amino acids will be cleaved to produce the mature polypeptide (FIG. 14; SEQ ID NO: 37). /--

Please delete the paragraph that appears at page 49, lines 14-27, and substitute the following paragraph in place thereof.

C¹² --] The amplification products HOMP5'/SO-E and SO-F/HO3'AN will be purified from agarose gel following separation by electrophoresis, and will be mixed, and subjected to further amplification using primers HOMP5' and HO3'AN. The resulting product encodes amino acids 1-52 and 211-591 of wild-type NhhA of PMC21. This amplification product will be subjected to restriction digestion with *EagI* and *NcoI*, and cloned into pCO14K. This recombinant molecule contains regions C1, C4, V4 and C5 thus deleting regions V1-3 and C2-3. The nucleotide sequence of the open reading frame is shown in FIG. 8 and SEQ ID NO: 31, and the predicted polypeptide sequence derived from this nucleotide sequence is shown in FIG. 8 and SEQ ID NO: 26. Analysis of the predicted amino acid sequence using the computer